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ORAL ADMINISTRATION OF PEPPERMINT IN WISTAR ALBINO RATS: Memory Boosting and Regaining

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ABSTRACT

The studies on peppermint aroma fluence on cognition are numerous. However the knowledge about oral consumption of peppermint and cognition was inadequate. Hence the present study was undertaken to find out the effect of oral administration of peppermint spices in memory boosting and memory regaining on adult wistar rats. Here we investigate the influence of oral intake of peppermint on behavioral task performance by using T-maze and radial arm maze and physiological measures relative to a milk control group. We have observed significant memory boosting and memory regaining effects of peppermint when administered orally. This effect may be due to improvement of the blood flow to the brain and increasing the concentration power. Hence we recommend further research in this area by investigating compound metabolism to optimize quantification of memory performance following peppermint ingestion.

Key words: Memory boosting, Memory retention, peppermint ingestion.

INTRODUCTION

Learning is acquisition of knowledge or skills as consequence of experience and memory is the retention of acquired knowledge for later recall.[1] Learning and memory are vital attributes of human intelligence. Memory experts often speak of different stages or types of memory: the three most basic types of memory evident in humans and possibly in laboratory animals are sensory, short term, and long term memory. The process of transferring short-term memory traces into long-term memory stores is known as consolidation.[10]

Spatial learning and memory is the ability to encode, store and retrieve information about location, configuration and routes. For exploration of an environment, animal utilizes internal and external cues. Internal cues are self generated movement cues that provide information about the distance and the direction from the start position. Many mazes have been used to test hippocampal function. The Radial Arm Maze (RAM), T-maze and water maze are perhaps the most used among them.[2] Hippocampus is one of the most essential integrating centers of the nervous system. It belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation.[3]

Numerous medicinal plants are mentioned in ancient Indian literature on cognition enhancers as well as ayurvedic treatments.[7] Existing literature suggests that consumption of natural compounds can improve memory. Fish oils, extra virgin olive oil, and antioxidant-rich foods such as spinach and berries have been shown to improve working memory and reduce loss of established memory.[14] Mint is one of the most famous natural herbs used for its analgesic, anti-inflammatory, antispasmodic, antioxidant, and vasoconstrictor effects.[11]

The aroma of peppermint has been found to enhance memory and alertness.[8] Peppermint is a natural remedy for boosting memory, focus and concentration.[9]

The knowledge about oral consumption of peppermint and memory is inadequate.[13] Hence the present study was undertaken to find out the effect of oral administration of peppermint spices in memory boosting and memory regaining on adult wistar rats.

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Materials and methods

Subjects

A total of 36 male and female wistar albino rats were used for this study. They were housed in groups, in propylene cages in an acclimatized (25-27^{o}C) room and were maintained on a 12hr light / dark cycle. Food

and water was given ad libitum until they aged 30 days at the beginning of the experiment. They were randomly assigned into control and peppermint groups with 18 rats in each group. Peppermint was administered to Peppermint group and milk without peppermint was given to control group.

Materials and Methods

T-maze

T-maze is made of wood with smooth polished surface. It consists of a stem (35 x 12 cm), a choice area (12 x 12 cm) and two arms (35 x 12 cm); at the end of each arm contain a food well. The sidewalls are 40 cm high. The choice area is separated from the arms by a sliding door.

Radial arm maze

Radial arm maze is made of Plexiglas; consist of eight equally spaced arms radiating from an octagonal central platform. Each arm was having a length of 56.2cm, width of 7.9 cm and height of 10 cm. The entire maze is elevated 80 m above the floor for easy locating of spatial cues by rats.

Peppermint extract

Peppermint (Mentha × piperita, also known as M. balsamea Willd.) is a hybridmint, a cross between watermint and spearmint. *Mentha piperita* leaves were washed, weighed (100g/L), and triturated with water in a blender for 7 minutes. The juice was filtered and frozen in an amber flask. Each flask was thawed daily at ambient temperature two hours prior to administration

Pharmacological drug

Buscopan® tablets manufactured by Cadila Healthcare limited, is used in the present study. Each Buscopan tablet contained Hyoscine (scopolamine) Butylbromide I P 10 mg and excipients (q. s.). The tablets were powdered and mixed with sterile 0.9% w/v normal saline. It was administered to the rats as intraperitoneal injection at a dose of 1 mg / Kg. Deficits in short-term memory have been reported following scopolamine administration in monkeys and in humans.[6] Scopolamine appears to be less disruptive to long-term memory storage than to short-term memory.[4] Scopolamine is a muscarinic antagonist structurally similar to the neurotransmitter acetylcholine and acts by blocking the muscarinic acetylcholine receptors and is thus classified as an anticholinergic.[5]

Experimental design

The rats in the peppermint group were given 5mg/kg body weight of peppermint leaf extract orally for 30 days continuously. The control rats were given equal quantity of milk for 30 days without peppermint extract. All the rats were fed with pellets and water mixed with B complex tonic liberally in these 30 days. After 30 days, the rats were starved for 48 hours and after 48hours the behavioural task is performed on T-maze and radial arm-maze for acquisition.

This task is continued till we recorded full score without any error. Now ten days gap was given for the retention of the task. In these ten days only pellets and water mixed with B complex tonic was given to both the groups. On eleventh day behavioral task is performed on T-maze and radial arm-maze and number of trials required to get full score is recorded in both the groups to test memory boosting effect of peppermint. From the next day we have started administration of scopolamine intraperitoneally to both the groups to cause partial amnesia. This procedure continued for 9 days. Scopolamine administration was done at 10 am daily. Only water mixed with B complex tonic is given to both the groups during this 9 days. From tenth day administration of scopolamine is stopped and peppermint is administered to peppermint group where milk without peppermint is given to the control group. This procedure continued for 30 days and food and water mixed with b complex was given to both the groups during these 30 days. On 31st day behavioral task is performed on T-maze and radial arm maze in both the groups for acquisition and number of trails required to get the full score is recorded. Now ten days gap is given where only food and water mixed with B complex is given to the rats in both the groups. On eleventh day behavioral tasks were performed on both the mazes to test the retention in both the groups and number of trails required to get the full score is recorded. The memory score was calculated by taking the difference between the number of trials required for acquisition test and number of trials for retention test**.**

The body weight was maintained at 85% of the original body weight, throughout experiment. Behavioral experiments were conducted in the same room with the same allocentric cues, such as doors, windows.

T-maze task

This was analogous to non-matching to sample task, where the rat was rewarded only if the current choice doesn't match the previous one. As reward is used it can also be considered as a learned alternation procedure. In the orientation phase, the starved rats were allowed to spend 10 minutes / day for three days in the T-maze and trained to collect food pellet from the food wells.

During the acquisition test, all the rats were given six trials / day with an inter trial interval of one hour. Each trial consists of four sample and choice run. In the sample run, the rat was placed at the start end

of the T-maze stem. Allowed to move towards one arm and collect the food pellet, while keeping the sliding door of other arm closed. In the choice run, the rat was placed at the start end of stem and both arms were kept open. If the rat visits the same arm as that of sample run, it was recorded as error and the rat was not rewarded with food. Instead, if the rat visits the alternate arm, it was recorded as correct score and the rat was allowed to eat food pellet (reward) in the food well. There was an interval of 30s between each run. Score was given for alternate selection of arm during choice run and a maximum score of '4' can be obtained per trial.

Radial arm maze task

The rats was placed in the centre of the maze and allowed to freely explore the maze for 15 minutes on the first day. The rats were required to take the food pellets from each arm without making a re-entry into the arm already visited. The trial was terminated when the animal takes the food reward from all the eight arms or after 10 minutes if all the eight arms were not visited. Correct sore was give when the visits an arm and collects the food reward, and a maximum score of '8' can be attained per trial. When a rat reenters an already visited arm it was taken as a working memory error.

Data analysis

The analysis of data was done by SPSS 20.0. The Independent Samples t-test procedure compares means for two groups of cases. Ideally, for this test, the subjects should be randomly assigned to two groups, so that any difference in response is due to the treatment (or lack of treatment) and not to other factors.

Ethical approval

The study protocol was approved by Institutional Ethics Committee of Little Flower Medical Research Centre, Angamaly.

Results

Data of mean trials of acquisition and retention in control were listed in Table 1.

Table 1
Number of mean trials of acquisition and retention in control and coriander (Radial arm maze memory boosting).

Parameter	Control group	Peppermint group	*p**
Acquisition	27.33±3.01	12.17±2.32	<.001
Retention	17.00±2.37	6.83±1.47	<.001

*significance at $p < 0.05$

The number of mean trials of acquisition in control group is 27.33±3.01 and in peppermint is 12.17±2.32, which indicates that peppermint group is having more memory boosting effect than control group. This is statistically significant (p<0.001). The number of mean trials of retention of control group is 17.00±2.37 and in peppermint group is 6.83±1.47, which indicates that group coriander is having more memory boosting effect than control group. This is statistically significant (p<0.001).

Table 2
Number of mean trials of acquisition and retention in control and peppermint (Radial arm maze memory regaining)

parameter	Control group	Peppermint group	*p**
Acquisition	40.83±1.94	18.67±2.16	<.001
Retention	20.50±1.87	11.83±2.14	<.001

*significance at $p < 0.05$

The number of mean trials of acquisition in control group is 40.83±1.94 and in peppermint group is 18.67±2.16, which indicates that peppermint group is having memory regaining effect. This is statistically significant (p<0.001). The number of mean trials of retention of control group is 20.50±1.87 and in peppermint group is 11.83±2.14, which indicates that peppermint group is having memory regaining effect. This is statistically significant (p<0.001).

Table 3
Number of mean trials of acquisition and retention in control and peppermint (T-maze memory boosting)

Parameter	Control group	Peppermint group	*p**
Acquisition	13.50±3.27	6.67±0.82	0.001
Retention	9±2.61	4.33±0.52	0.002

*significance at $p < 0.05$

The number of mean trials of acquisition of control group is 13.50±3.27 and in peppermint is 6.67±0.82, which indicates that peppermint group is having more memory boosting effect than control group. This is statistically significant (p<0.001).

The number of mean trials of retention of control group is 9±2.61 and in peppermint group is 4.33±0.52, which indicates that peppermint group is having more memory boosting effect than control group. This is statistically significant (p<0.002).

Table 4 reveals that the number of mean trials of acquisition in control group is 24.17±3.66 and in peppermint group is 11.17±1.47, which indicates that peppermint group is having memory regaining effect. This is statistically significant (p<0.001). The number of

mean trials of retention in control group is 13.83±2.48 and in peppermint group is 8.00±0.63, which indicates that peppermint group is having memory regaining effect. This is statistically significant (p<0.001).

Table 4
No of mean trials of acquisition and retention in control and peppermint (T-maze memory regaining)

Parameter	Control group	Peppermint group	p^*
Acquisition	24.17±3.66	11.17±1.47	<.001
Retention	13.83±2.48	8.00±0.63	<.001

*significance at $p < 0.05$

DISCUSSION

It was reported that Peppermint aroma enhances memory.[12,17] Peppermint aroma produced a marked increase in word recall accuracy.[15] Consumption of peppermint does not mediate alertness or enhanced cognitive performance but improves concentration.[13] In contrast it was reported that chewing peppermint gum increases working memory and visual motor response.[16]

This mechanism would require pharmacological action, including compound absorption and subsequent neuronal action. We agree with this study as we have observed significant memory boosting and memory regaining effect of peppermint when administered orally. This effect may be due to improvement of the blood flow to the brain and increasing the concentration power.

Conclusion

We conclude that oral administration of peppermint is having memory boosting and memory regaining effects in rats. Hence we recommend further research in this area by investigating compound metabolism to optimize quantification of memory performance following peppermint ingestion.

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